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NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND INFLAMMATORY BOWEL DISEASE: CURRENT PERSPECTIVES

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Mechanisms underlying the gastric toxicity of nonsteroidal anti-inflammatory drugs (NSAIDs) have been extensively investigated, whereas those leading to intestinal damage are not completely understood. Several hypotheses have been put forward on the pathophysiology of intestinal damage by NSAIDs: enhanced intestinal permeability, inhibition of cyclooxygenase (COX), enterohepatic recirculation, and formation of adducts. The effects of COX-2 selective inhibitors, which appear to have better gastric tolerability when compared to nonselective NSAIDs, on normal and inflamed intestinal mucosa (as in Crohn's disease or ulcerative colitis) are still largely unexplored. If COX-2 inhibition plays a key role in suppressing the inflammatory process, recent evidence suggests that COX-2 products are involved in maintaining the integrity of intestinal mucosa, in the healing of gastrointestinal ulcers and in the modulation of inflammatory bowel disease (IBD). Animal models of intestinal inflammation have so far yielded conflicting results on the effects of COX-2 selective inhibitors on the intestinal mucosa. It is now clear that NSAIDs do not act through cyclooxygenase inhibition, but also have different targets such as nuclear factor-κB and/or peroxisome proliferator-activated receptors γ. The peculiar pharmacological profile of each compound may help to explain the different impact of each NSAID on the inflammatory process and on IBD. Notably, the salicylic acid derivative 5-ASA is widely used in the treatment of IBD and is believed to act through nuclear factor-κB inhibition. Although the use of COX-2 selective inhibitors remains contraindicated in patients with IBD, studying their effects on intestinal mucosa may offer new insights into their subcellulars mechanisms of action and open new avenues for the development of novel therapies for IBD.

KEY WORDS: inflammatory bowel disease, cyclooxygenase, intestinal mucosa, side effects, cytoprotection.

BACKGROUND

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most prescribed drugs because of their anti-inflammatory and analgesic properties. However, their use is associated with an elevated risk of damage to the gastrointestinal mucosa and related complications [1–4]. Henry *et al.* [5] studied the relative risk of gastrointestinal complications using a meta-analytical approach: the meta-analysis showed wide differences among individual drugs, although some of the differences may be explained by dose.

Because inhibition of prostaglandin synthesis is central to both the beneficial and toxic effects of NSAIDs, they have been regarded as a double-edged sword. The discovery that cyclooxygenase (COX) exists in two isoforms, [6–8] with COX-2 being the primary isoform at the site of inflammation, led to hypothesize that inhibition of this isoform accounts for the therapeutic benefits of NSAIDs as anti-inflammatory agents whereas inhibition of COX-1 is responsible for the adverse effects on the gastrointestinal tract. On this basis, intense efforts were made to develop selective COX-2 inhibitors [9].

In the past decade, it has been recognized that NSAIDs can damage not only the upper gut (stomach and duodenum [10]), but also lower segments of the gastrointestinal tract (Table I). The mechanisms of injury to the small bowel and colon are not as well characterized as those in the upper gut. In particular, the impact of COX inhibition on the intestinal mucosa in inflammatory bowel disease (IBD) is controversial, all the more so because of the

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Table I
Type, severity, and localization of NSAIDs toxicity [16, 24]

Effects on upper gastrointestinal tract
Mild side effects:
Dyspepsia
Gastrointestinal erosions (stomach > duodenal bulb)
Moderate side effects:
Iron-deficiency anaemia
Gastrointestinal ulcers (stomach and intestine)
Scarring (antrum and duodenal bulb)
Serious complications:
Severe gastrointestinal bleeding (stomach > duodenal bulb > oesophagus, small and large intestine)
Acute perforation (duodenal bulb > colon)
Gastric outlet obstruction
Effects on small intestine
NSAIDs enteropaty:
Small intestinal bleeding
Protein losing enteropathy
Bile acid malabsorption
Perforation
Ulcers
Strictures
Effects on colon
Colitis:
Nonspecific
Eosinophilic colitis
Pseudomembraneous colitis
Collagenous colitis
Ischemic colitis
Appendicitis
Colonic bleeding and perforation
Relapse of IBD
Diverticular complications

recent finding that some NSAIDs may, in certain conditions, have a proinflammatory effect [11].

There is now growing evidence that NSAIDs act not only by inhibition of cyclooxygenase pathway, but also modulate cyclooxygenase-independent signal transduction pathways, which may be involved in both the anti-inflammatory and anti-tumor activity of these drugs [12].

Interestingly, the salicylic acid derivative 5-aminosalicylic acid (5-ASA) is widely used in the treatment of IBD. Its action is due at least in part to inhibition of activation of the transcription factor NF-κB [13].

The aim of the present review is to focus on mechanisms responsible for damage to the intestinal mucosa by NSAIDs, discussing the possible impact of COX inhibition in IBD.

MECHANISMS LEADING TO INTESTINAL DAMAGE

An enteropathy can be detected in 20–60% of NSAIDs recipients, depending on the method used for the diagnosis [14, 15]. Although most of the clinical evidence on the intestinal adverse effects of NSAIDs comes from case reports [16], several experimental studies in animals allowed to put forward different hypotheses to explain the possible mechanisms of enteric toxicity.

Enhanced intestinal permeability

NSAIDs appear to cause specific damage to enterocyte mitochondria during absorption by uncoupling oxidative phosphorylation. The subsequent reduction in ATP levels in turn results in loss of integrity of intercellular junctions with increased intestinal permeability. Thus, the mucosa is exposed to luminal aggressive factors, such as bile, proteolytic enzymes, and bacterial degradation products [16].

Small intestinal permeability has been used to quantify mucosal alterations induced by NSAIDs. These permeability changes have been detected by oral administration of probes such as ^{51}Cr-EDTA [17], lactulose [18], cellobiose [19], and polyethylenglycol [20]. The 4-day faecal excretion of ^{111}In-labeled white cells has also been used to assess NSAIDs enteropathy, although this test involves exposure to radiation and is demanding on patients [16]. More recently, a simple faecal test has been proposed to diagnose NSAIDs enteropathy [21]. Single stool faecal calprotectin concentrations were found significantly higher in NSAIDs recipients than in controls. Calprotectin is a calcium binding protein found in neutrofils, monocytes, and macrophages: it resists metabolic degradation and can be measured in faeces, hence its use as a quantitative marker of intestinal inflammation. Interestingly, 44% of NSAIDs recipients had elevated faecal calprotectin and 20% of these had comparable levels of inflammation to those reported in patients with IBD [21].

Recently, Smecuol *et al.* [22] have compared the effect of four different NSAIDs at equieffective doses on gastrointestinal permeability and found that intestinal permeability was significantly increased by naproxen, indomethacin, and meloxicam, but not by celecoxib. Whether other selective COX-2 inhibitors share this favorable profile awaits confirmation.

COX inhibition

While the development of a highly selective COX-2 inhibitor seems a rational approach to obtain gut-sparing NSAIDs, the emerging role of prostaglandins as modulators of mucosal defense in situations in which the mucosa is inflamed raises several issues that deserve consideration [23].

Growing evidence indicates that the classical COX hypothesis is oversimplistic: both COX-1 and COX-2 are involved as a constitutive and as an inducible enzyme in inflammation and cytoprotection [24]. Indeed, two recent studies in rats have shown that, whereas selective inhibition of COX-1 or COX-2 is not ulcerogenic, combined inhibition of COX-1 and COX-2 induces severe lesions in the stomach and small intestine suggesting an important contribution of COX-2 to the maintenance of gastrointestinal mucosal integrity [25, 26]. Furthermore, COX inhibition during ulcer healing seems to be detrimental [24], as indicated by the fact that indomethacin or diclofenac delay gastric ulcer healing both in experimental animals and humans [27, 28].

Studies of COX-2 mRNA and protein expression demonstrated that in rats COX-2 expression is strongly upregulated in the margins of healing gastric ulcers [29]. At the site of ulceration, COX-2 appears to be the primary contributor to prostaglandin synthesis. COX-2 thus appears to represent the second line of defense, which is activated during ulcer healing to compensate for the temporary loss of COX-1 occurring in the mucosa adjacent to the ulcer and assisting COX-1 in safeguarding gastric mucosal integrity. Interference of the selective COX-2 inhibitor L-745 337 with healing dynamics of experimental gastric ulcers was demonstrated in the rat cryoulcer model [30]. Similar results were obtained with the selective COX-2 inhibitor NS-398 in mice and rats [31, 32].

Thus, although COX-2 selective inhibitors have been shown to cause markedly less gastrointestinal injury than standard NSAIDs in healthy animals and humans [33, 34], they need to be assessed in conditions of preexisting gastrointestinal inflammation.

The role of eicosanoids in the intestinal inflammatory process is not completely understood: several eicosanoids are increased in IBD and a positive correlation exists between tissue levels of these eicosanoids and disease activity. COX-1 expression is detected in both normal and inflamed gastrointestinal mucosa; in contrast, COX-2 is expressed in epithelial cells in the upper portions of the crypts and on the surface in Crohn's colitis and ulcerative colitis, in villous epithelial cells in Crohn's ileitis and is not detectable in the epithelium of the normal ileum or colon. These findings suggest that COX-2 expression is induced by inflammatory mediators in the different portions of the epithelium in Crohn's and ulcerative colitis and in Crohn's ileitis [35, 36].

Nonselective NSAIDs suppress prostaglandin synthesis and can exacerbate some experimental models of colitis [37]. COX-2 is upregulated in the colonic mucosa in both experimental and human colitis [35] and appears to have a beneficial effect in healing experimental colitis. On the other hand, COX products are fundamental for the inflammatory process and COX-inhibition could be beneficial [13].

The effects of NSAID therapy in patients with IBD have been recently evaluated by Bonner *et al.* [38]. This retrospective study failed to demonstrate a correlation between NSAIDs use and likelihood of active IBD, contrary to prior studies. Thus, there may be subsets of IBD patients who can tolerate NSAIDs with less likelihood of an exacerbation of IBD [39].

There is evidence that prostanoids produced via COX-1 contribute to inflammation, pain, and fever, and that prostanoids derived from COX-2 exert immunomodulatory and cytoprotective effects [40] and contribute to the muscle hypercontractility that persists after bacterial infection [41].

More recently, McCartney *et al.* [42] demonstrated that the elevated production of prostanoids in human IBD is dependent upon the activity of COX-2.

Enterohepatic recirculation

Reuter *et al.* [43] suggested that NSAID-induced small intestinal injury in rats is largely dependent on the degree to which the NSAID undergoes enterohepatic recirculation. Initial changes in epithelial permeability, probably caused by a topical irritant action of the NSAID, do not necessarily lead to frank ulceration. With repeated exposure of the intestine to the NSAID, as it is excreted in to the bile, further epithelial injury occurs and is exacerbated by elevated numbers of luminal bacteria. The elevation of enteric bacterial number occurs only with NSAIDs that undergo enterohepatic recirculation. According to this view, systemic suppression of prostaglandin synthesis by the NSAID does not play an important role in the pathogenesis of small intestinal ulceration and the better intestinal tolerability of moderately selective COX-2 inhibitors (e.g. etodolac and nabumetone) is attributable to the lack of enterohepatic recirculation rather than to COX-2 selectivity. If these findings are confirmed in humans, important new strategies for the development of gut-sparing NSAIDs may be followed.

Formation of drug enterocyte adducts

Covalent binding of a toxicant to target macromolecules is considered a key mechanism of cell injury [44]. Formation of adducts by reactive diclofenac metabolites with hepatic macromolecules has been described in the liver of rodents; hepatic uridine-5′-diphosphoglucuronosyltransferase (UDPGT) is known to biotransform diclofenac and other NSAIDs into acyl ester glucuronides [45]. Atchison *et al.* [46] have investigated, first, the hypothesis that formation of adducts between a reactive metabolite of diclofenac and enterocyte constituents is a causal factor in diclofenac-induced enteropathy in rats. This hypothesis was substantiated by the finding that adduction occurs in proximity to the site of injury, adduction precedes temporally ulcer formation and its entity corresponds to the magnitude of injuries.

Damage by adducts depends on enterohepatic recirculation of NSAID: indeed, neither adducts nor ulcers were detected in diclofenac-treated animals when their bile was externally drained or their bile ducts were ligated.

COX INHIBITORS AND IBD

Animal models of IBD support the contention that eicosanoids are involved in modulating tissue inflammation [47], but the studies on the impact of COX-2 inhibition on animal models of colitis are widely dissonant (Table II). In accordance with the central role of eicosanoids in maintaining the inflammatory process, Karmeli *et al.* [48] demonstrated that COX-2 inhibitors have a beneficial effect on experimental colitis in rats, acting by reducing colonic eicosanoids generation. In contrast, Reuter *et al.* [49] demonstrated that selective inhibition of COX-2 can result in exacerbation of inflammation-associated colonic injury, in accordance

Table II
Effect of some NSAIDs in animal models of colitis

Species	*Induction of colitis*	*Drugs/dose*	*Effect*	*References*
Male Wistar rat	Trinitro-benzene sulfonic acid (TNBS) 50 mg kg^{-1}	Diclofenac 10 mg kg^{-1}, Naproxen 5 mg kg^{-1}, Nabumetone 25 or 75 mg kg^{-1}, Etodolac 10–50 mg kg^{-1}, L 745 337 1–5 mg kg^{-1}	Exacerbation of colitis (evaluation of histological damage, prostaglandin synthesis, and thromboxane concentration *in vivo*)	[49]
Male Sprague–Dawley rat	Trinitro-benzene sulfonic acid (TNBS) 50 mg kg^{-1}	NS 398 1,10,100 mg kg^{-1}, SC-58125 1,10,100 mg kg^{-1}, PD-138387 1,10,100 mg kg^{-1}	No benefit (evaluation of histological damage, colon weight, mycloperoxidase activity, synthesis of PGE_2)	[50]
Male Sprague–Dawley rat	2 ml of acetic acid (5%) or 0.1 ml of iodoacetamide (3%)	Nimesulide 2 × 10 mg kg^{-1}, SC 236 6 mg kg^{-1}	Amelioration of colitis (evaluation of histological damage, MPO and NOS activity, synthesis of PGE_2)	[48]

with the detrimental effect of NSAID on gastric ulcer. Finally, Lesch *et al.* [50] has shown that selective COX-2 inhibitors are unable to produce any significant beneficial effect in experimental colitis.

Notably, in these studies different models of experimental colitis, different compounds and different doses were used.

Only one study is available on the effect of a selective COX-2 inhibitor on colonic mucosa in human IBD [42]. This study compared the effects of a highly selective COX-2 inhibitor, L-745 337 [51] and of a traditional NSAID, indomethacin, on the release of prostanoids from colonic mucosal biopsies obtained from both patients with IBD (ulcerative colitis or Crohn's disease) and from normal controls. The marked increases in tissue prostanoid production typical of active IBD are as sensitive to inhibition by L-745 337 as they are to indomethacin. The elevated production of prostanoids in IBD is therefore dependent upon the activity of COX-2. This is an important finding, since it is widely accepted that prostanoids of the E and I class are generally protective within the human gastrointestinal tract [52]. The authors concluded that selective COX-2 inhibitors, by reducing the production of prostanoids to the same extent as a traditional NSAID, would reduce the cytoprotective effects of locally produced PGE_2 and PGI_2 and so, like a nonselective NSAID, aggravate mucosal damage.

CURRENT PERSPECTIVES

An aspect that is often overlooked is that every NSAID (whether or not it is a selective COX-2 inhibitor) is endowed with a range of pharmacological activities that may lead to significant differences in the effects of an individual agent on the inflamed mucosa. For instance, the salicylic acid derivative 5-ASA is widely used in the treatment of IBD, and its anti-inflammatory action is due to inhibition of activation of transcription factor NF-κB [13].

NF-κB designates a group of transcription factors consisting of different proteins: in nonstimulated cells, it is sequestered in the cytoplasm as a latent, inactive complex bound to inhibitory proteins termed IκB. Many different stimuli (cytokines, lipopolysaccharides, bacterial, and viral infections, activators of protein kinase C, oxidants) activate NF-κB by phosphorylation of IκB via IκB-kinase. The action of NF-κB in the nucleus activates the transcription of a wide spectrum of proinflammatory genes with consequent production of cytokines, adhesion molecules and enzymes involved in inflammation [13]. Inhibitors of NF-κB activation have been shown to be very potent anti-inflammatory agents. Some NSAIDs, such as sodium salicylate, sulindac, ibuprofen, and flurbiprofen have been unequivocally shown to cause anti-inflammatory and anti-proliferative effects independent of cyclooxygenase inhibition, through an action on NF-κB [12]. In contrast, celecoxib did not inhibit but activate NF-κB, suggesting that its cyclooxygenase-independent actions may also differ from those of nonselective agents. It is not yet known whether this NF-κB activating effect is shared by all COX-2 selective agents or whether it is a special feature of celecoxib [53].

Interestingly, some other NSAIDs, such as indomethacin (which does not inhibit NF-κB), diclofenac, ibuprofen, fenoprofen, and flufenamic acid, bind and activate (with different affinity) PPAR-γ (peroxisome proliferator-activated receptors) [11, 12].

PPAR-γ are ligand-activated transcription factors belonging to the nuclear receptor family. Known at the beginning as regulators of lipid and lipoprotein metabolism, more recently PPARs have been identified as important players in the metabolism of lipid-derived inflammatory mediators and in inflammation-related disorders, such as atherosclerosis and IBD, acting principally by preventing the activation of NF-κB [54–56]. The PPAR-γ is highly expressed in the colonic mucosa and its activation has been reported to protect against colitis. Recently, PPAR-γ agonists have been reported to attenuate colitis in a murine model in which inflammation was induced by administration of dextran sodium sulfate [55]. This observation suggested that PPAR-γ activators may be useful in the treatment of patients with IBD. To activate transcription, PPAR-γ requires heterodimerization with the retinoid X receptor (RXR). The RXR/PPAR-γ heterodimers are

permissive to activation by both PPAR-γ and RXR ligands. More recently, Desreumaux *et al.* [56] investigated the potential effects of both PPAR-γ and RXR in an experimental animal model in which colitis was induced by intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS). These authors, consistent with work of Su *et al.* [55], showed that administration of PPAR-γ agonists, such as rosiglitazone or troglitazone attenuated the inflammatory response in TNBS-induced colitis. These results confirm that PPAR-γ ligands have anti-inflammatory effects in the intestine. Furthermore, simultaneous administration of both PPAR-γ and RXR ligands had a markedly synergistic beneficial effect on colitis, enabling a significant dose reduction for each agonist. These data suggest that the synergistic anti-inflammatory effect of RXR and PPAR-γ agonists could be beneficial in a clinical setting, as it might avoid adverse events often encountered when these agonists are used in monotherapy at higher doses and thus could represent an attractive therapeutic strategy for the treatment of IBD. It should be noticed that most of the NSAIDs so far investigated behave as partial PPAR-γ agonist and therefore they have the potential to reduce the beneficial effects of a full agonist [11].

CONCLUSIONS

From the preceding paragraphs, it emerges that the mechanisms leading to intestinal damage by NSAIDs are different from those underlying the gastric damage. Moreover, the two different isoforms of COX seem to play different roles in maintaining homeostasis of gastrointestinal mucosa and in the process of gastrointestinal ulcer healing.

The impact of COX-2 selective inhibitors on IBD is currently controversial: on one hand, COX inhibition is fundamental for the modulation of the inflammatory process; on other hand, COX-2 inhibition seems to have a detrimental role in ulcer healing.

It is now clear that classifying NSAIDs on the basis of their COX-1/COX-2 selectivity is simplistic because of the wide spectrum of pharmacological activities possessed by these compounds. Knowledge of the full range of activities of each NSAID (COX selectivity, action on NF-κB or PPAR-γ) will help to clarify its therapeutic and safety profile. Gaining insight into the cyclooxygenase-independent effects may also open new avenues for the development of new drugs for the treatment of IBD.

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